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and gene therapies. Here, to explore a potential of pH-sensitive polymer-liposome complexes for the tumor-specific combinatorial delivery of anticancer agents and siRNA, conventional liposomes (ConL), polymer-liposome complexes (PLC) and polymer-cationic liposome complexes (PCLC) were prepared. Pluronic® P104-based multiblock copolymer (MBCP-2) was included as pH-sensitive polymer. Physicochemical properties, release under different pH, cytotoxicity and *in vitro* cellular uptake of DOX-loaded liposomes were investigated. From the release test, an acidic pH was determined to be an important factor for release from the PLC vehicles. The novel PLC vehicle itself showed low cytotoxicity demonstrating suitable viability. Observing cellular uptake of DOX by confocal microscopy imaging, a greater amount of DOX was delivered to cells with the pH-sensitive polymer-anchored vehicles than that with free DOX and ConL. It was verified that the novel vehicles could effectively deliver both DOX and GFP-siRNA. Novel pH-sensitive PCLC have a potential for targeted therapy of anticancer agents and gene therapy under acidic tumor microenvironment.

575. Chitosan/siRNA Nanoparticles Demonstrate Superior Silencing Efficiency and Lower Toxicity Compared To Lipid Nanoparticles

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Purpose: Nanoparticles (NP) using specific chitosans (CS) have been developed for efficient delivery of siRNA, *in vitro* and *in vivo*. Safety of such systems as defined by the lack of both systemic and chronic toxicity is the most critical aspect as per FDA, EMEA and ICH guidelines. Development of CS NPs for clinical use requires comparative toxicity profiles. A fractional factorial design (FFD) was used to screen factors affecting toxicity to select non-toxic formulations with optimal biological activity for *in vivo* studies.

Methods: A combinatorial approach was adopted to evaluate the *in vitro* toxicity of NP using the H1299 cell line. A 2^{5-1} FFD was used to screen for the effects of chitosan molecular weight (Mn), amine to phosphate ratio (N:P), presence of serum at time of transfection and siRNA concentration ([NA]) on NP toxicity. Post transfection metabolic impairment induced by NP delivering anti-eGFP siRNA was assessed after 4 and 48 hours using alamarBlue assay. Quantification of NP genotoxicity and evaluation of silencing efficiency (SE) were performed using the alkaline comet assay and flow cytometry 48 hours post-transfection respectively. Results relative to non transfected cells were compared to DharmaFECT (DF) and Doxorubicin.

Results: CS-NP induced significantly less toxicity (0-25% vs 60-70% cell death) than DF 48 hours post-transfection. Low molecular weight chitosans (LMWCS 10kDa) induced a slightly higher toxic effect than their high molecular weight counterparts (HMWCS 150kDa). Serum proteins showed no influence on cytotoxicity. NP prepared in diluted versus concentrated regimes (0.05 vs 0.2µg/L) showed significantly different sizes but no influence on toxicity. Hence NP size did not correlate with toxicity. For a final siRNA concentration of 100nM/well, the increase in the quantity of chitosan (0.33-3.3µg/well, i.e. N:P of 5 and 60) did not affect the toxicity profile observed either with LMWCS or HMWCS. [NA] up to 500 nM showed no increase in toxicity regardless of the N:P ratio. These results suggest that cells readily tolerate siRNA and chitosan quantities near 7µg/mL and 165µg/mL, respectively. Silencing efficiency reached ~50-70%. Interestingly, an increase in chitosan charge density (DDA from 92 to 98%) resulted in higher toxicity for LMWCS, reminiscent of the mitochondrial induced toxicity of Polyethylenimine but only for chitosan of the highest DDA. Genotoxicity assessment using the comet assay corroborated the results obtained with alamarBlue. DF, a lipid formulation used as positive control for SE, demonstrated high toxicity compared to chitosan in all tests.

Conclusion: Chitosan is a superior choice for preparation of NP compared to lipids owing to its high efficiency (>60%) combined with very low toxicity. Assessment of apoptotic pathway activation for LMWCS by pathway focus qPCR gene profiling arrays is ongoing. Finally, cell cycle and DNA damage response pathway profiling will reveal mechanistic insights into toxicity pathways.

576. Formulation Optimization of PEI-DNA Complexes for the Efficient Gene Delivery To Cultured HeLa Cells

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The purpose of this study is to examine the optimized formulation of the luciferase-expressing plasmid DNA complexed with the synthetic cationic polymer polyethyleneimine (PEI) that shows the highest transfection activity in HeLa cell. The PEI/DNA ratio that produced the highest luciferase activity upon transfection into HeLa cells was found to be around 5. Assuming that the size and shape of the PEI-DNA complex is one of the most determinative variables for the high transfection efficiency and that the mixing dynamics of the two macromolecules can have a profound effect in this regard, we examined the effect of the concentrations of PEI and DNA and the size of PEI on the transfection efficiency at N/P ratio=5. The highest transfection efficiency was observed when DNA (2 ug/ul) was mixed with 10mM PEI (25kD). Upon electron microscopic examination, the PEI-DNA complex assumed regular spherical shapes with 40-60 nm in size. We also examined the effect of serum and antibiotics and found that the best results were obtained at 0.5% FBS without any antibiotics. At optimal conditions the PEI-DNA complexes yielded 2-3 fold higher transfection efficiency than commercial liposomal agents. Our results should prove informative in formulating PEI-DNA complexes for optimal gene delivery to cultured cell lines and the optimized formulation of PEI-DNA complexes may be further improved to be adopted for efficient in vivo gene delivery.

577. DOTAP and MPEG-PCL Hybrid Nanoparticles for Cancer Gene Therapy

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Cationic nanoparticles show promise for applications in gene delivery. Commercial liposomal gene carriers have high transfection efficiency but strong cytotoxicity which is caused by the high content of cationic DOTAP in the liposome. Here, DOTAP is used to modify degradable monomethoxy poly(ethylene glycol)–poly(ε-caprolactone) (MPEG–PCL, MP) micelles in one step, creating the cationic self-assembled DOTAP and MPEG–PCL hybrid micelles (DMP). These micelles have a mean particle size of 46 nm and a zeta potential of +41 mV, and are able to bind plasmid DNA. The content of DOTAP in DMP micelles is as low as 10%. Compared with PEI25K (the gold standard), DMP micelles had higher transfection efficiency and lower cytotoxicity. DMP delivered survivin-T34A gene could inhibit the growth of C-26 colon cancer cells through inducing apoptosis in vitro. Intraperitoneal administration of DMP delivered Survivin-T34A gene efficiently inhibited the growth of abdominal metastatic C-26 colon cancer and the malignant ascites. These data suggest that DMP micelles are a novel gene carrier, and its delivery of survivin-T34A gene may have promising applications in colon cancer treatment.